Supporting Information

Synthesis and Biological Evaluation of D-gluconhydroximo-1, 5-lactam and Its Oxime-substituted Derivatives as Pharmacological Chaperons for the Treatment of Gaucher Disease

Experimental section

Cytotoxicity assay in wild-type human fibroblasts.

CCC-ESF-1 cells were seeded at a density of 4000 cells per well in 96-well plates and incubated overnight. The cells were treated with a range of concentrations from 100 to 3.125 µM at 37°C in 5% CO2 for 24h. Then 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT, 20 µL, 5 mg/mL in PBS; Sigma–Aldrich) was added to each well, and the mixtures were incubated for additional 4 h at 37°C. After removal of the medium, formazan was dissolved in DMSO (150 µL) and quantified by using a microplate reader (570 nm). Experiments were performed in triplicate. Herein, only show the results at 100 µM, the highest concentration in activation assays, and no cytotoxicity was observed (Table S1)

<table>
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<tr>
<th>Compound</th>
<th>% Cell inhibition</th>
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<td>1.19</td>
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<td>IFG</td>
<td>0.45</td>
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Table S1. Cytotoxicity of compounds 27-38, IFG and NN-DNJ at 100 µM in wild-type human fibroblasts.

General procedures. All reagents for synthesis were obtained from commercially sources and were of reagent grade. All solvents were available commercially, dried or freshly dried and distilled prior to use. Thin-layer chromatography (TLC) was performed on silica gel GF254 plates with detection using shortwave UV light (λ=254 nm) and staining with 10% phosphomolybdic acid in EtOH or a p-anisaldehyde solution (EtOH/p-anisaldehyde/AcOH/H2SO4, 135:5:4:1.5), followed by heating on a hot plate. Flash chromatography was performed with silica gel (100–200 mesh) with EtOAc/petroleum ether or EtOAc (or CH2Cl2)/MeOH or EtOAc/acetone/NH4OH/H2O as eluent. 1H and 13C NMR spectra were recorded on a Bruker AV 400 spectrometer at 400 MHz (1H NMR) and 100 MHz (13C NMR), using CDCl3 or CD3OD as solvents. Coupling constants are reported in Hertz. High-resolution mass spectra (HRMS) were obtained on a Varian QFT-ESI mass spectrometer.

2,3,4,6-tetra-O-benzyl-D-glucothionolactam (14). 2,3,4,6-Tetra-O-benzyl-D-gluconolactam 13 (2.56 g, 5.7 mmol) (obtained in eight steps from Methyl α-D-glucopyranose 12 using established protocols) and Lawesson’s reagent (3.47 g, 8.6 mmol) in toluene (100 mL) was stirred at room temperature for 36 h. The mixture was concentrated in vacuo and extracted with CH2Cl2 (150 mL) and washed with water (2×50 mL), the organic phase was collected and dried with anhydrous Na2SO4, the solvent was removed and the crude product subjected to flash column chromatography (petroleum ether/ethyl acetate = 5:1) to give 14 a white solid (2.91 g, 92%). 1H NMR (400 MHz, CDCl3): δ 7.39-7.41 (m, 2H), 7.24-7.35 (m, 16H), 7.13-7.14 (m, 2H), 5.02 (dd, J = 11.6, 2.0 Hz, 1H), 4.74 (d, J = 12.0, 2.0 Hz, 1H), 4.67 (d, J = 11.6 Hz, 1H), 4.58 (d, J = 11.6, 1.6 Hz, 1H), 4.41-4.48 (m, 4H), 4.35 (d, J = 11.6, 2.0 Hz, 1H), 3.85-3.90 (m, 2H), 3.61-3.64 (m, 1H), 3.55-3.59 (m, 1H), 3.35-3.40 (m, 1H); 13C NMR (100 MHz, CDCl3): δ 200.43, 137.45, 128.63, 128.51, 128.46, 128.44, 128.36, 128.24, 128.14, 128.05, 127.93, 82.54, 81.37, 78.43, 73.44, 72.82, 72.65, 72.57, 68.37, 56.01. HRMS (ESI): m/z [M+ H]+ calcd for C34H35NO5: 538.2594, found 538.2593
for 13; HRMS (ESI): m/z [M+Na]+ calcd for C\textsubscript{46}H\textsubscript{58}N\textsubscript{2}O\textsubscript{7}: 756.2179, found 756.2183 for 14.

2,3,4,6-tetra-O-benzyl-D-gluconhydroximo lactam (15). A mixture of 14 (2.91 g, 5.25 mmol), hydroxylamine hydrochloride (365 mg, 5.25 mmol) and sodium bicarbonate (365 mg, 5.25 mmol) in 50 mL methanol was refluxed at 75°C for 6 h. TLC analysis indicated the starting material was consumed completely, the mixture was concentrated in vacuo and extracted with 150 mL CH\textsubscript{2}Cl\textsubscript{2}, washed with 50 mL H\textsubscript{2}O and 50 mL saturated brines respectively, dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated in vacuo using rotary evaporator to get the crude product. The crude mixture was purified by column chromatography (petroleum ether/ethyl acetate = 5:1) to afford the product as a colorless oil (2.41 g, 83%).

\begin{align*}
\text{1H NMR (400 MHz, CDCl}_3) & \delta 7.38-7.28 (m, 18H), 7.16-7.14 (m, 2H), 4.76 (d, \ J = 12.0 Hz, 1H), 4.64-4.44 (m, 6H), 4.41 (d, \ J = 11.6 Hz, 1H), 3.78-3.75 (m, 1H), 3.70 (dd, \ J = 9.2, 2.4 Hz, 1H), 3.53 (dd, \ J = 9.6, 3.7 Hz, 1H), 3.48 (dd, \ J = 9.6, 7.2 Hz, 1H). \\
\text{13C NMR (100 MHz, CDCl}_3) & \delta 150.76, 137.79, 137.68, 137.55, 137.44, 128.62, 128.58, 128.50, 128.46, 128.22, 128.11, 128.00, 127.96, 81.55, 80.26, 73.47, 73.28, 72.47, 72.01, 71.26, 69.09, 51.75. HRMS (ESI): m/z [M+H]+ calcd for C\textsubscript{34}H\textsubscript{35}NO\textsubscript{4}S: 576.2179, found 576.2183.
\end{align*}

General procedure for the preparation of 16-26. To a stirred solution of 15 (100 mg, 0.18 mmol) and trimethylamine (47 µL, 0.36 mmol) in 5 mL of dry THF at 0°C was added a solution of various of substituted isocyanate (0.22 mmol) in 1 mL of dry THF. The reaction was allowed to warm to room temperature and was stirred for 8 h. The mixture was concentrated in vacuo and extracted with 50 mL, subsequently washed with 20 mL 1M HCl and 20 mL saturated sodium bicarbonate, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. The solution was concentrated under cacuum and purified by flash column chromatography (petroleum ether/ethyl acetate = 10:1 or 15:1) to afford the desired oxime substituted of 2,3,4,6-tetra-O-benzyl-D-gluconhydroximo lactam.

\begin{align*}
\text{O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-phenylcarbamate (16) A white solid (91.2 mg, 75%).} \\
\text{1H NMR (400 MHz, CDCl}_3) & \delta 7.49 (d, \ J = 8.4 Hz, 1H), 7.37-7.27 (m, 20H), 7.11-7.14 (m, 1H), 4.76 (d, \ J = 12.0 Hz, 1H), 4.61-4.45 (m, 6H), 4.34 (d, \ J = 11.6 Hz, 1H), 4.08 (s, 1H), 3.96 (s, 1H), 3.77 (m, 1H), 3.66 (dd, \ J = 9.6, 2.4 Hz, 1H), 3.58 (dd, \ J = 9.6, 3.6 Hz, 1H), 3.49 (dd, \ J = 9.6, 6.4 Hz, 1H). \\
\text{13C NMR (101 MHz, CDCl}_3) & \delta 152.55, 151.43, 137.57, 137.44, 137.40, 137.31, 137.16, 129.11, 128.63, 128.60, 128.45, 128.15, 128.13, 128.08, 127.95, 124.02, 119.60, 81.56, 79.94, 73.50, 73.31, 72.56, 72.03, 71.07, 68.41, 51.93. HRMS (ESI): m/z [M+H]+ calcd for C\textsubscript{6}H\textsubscript{12}N\textsubscript{2}O: 672.3074, found: 672.3075.
\end{align*}

\begin{align*}
\text{O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-Naphthylcarbamate (17) A white solid (97.4 mg, 75%).} \\
\text{1H NMR (400 MHz, CDCl}_3) & \delta 8.03 (d, \ J = 7.6 Hz, 1H), 7.93-7.83 (m, 2H), 7.68 (d, \ J = 8.4 Hz, 1H), 7.55-7.43 (m, 3H), 7.43-7.21 (m, 18H), 7.18-7.06 (m, 2H), 4.82 (d, \ J = 12.0 Hz, 1H), 4.67-4.43 (m, 6H), 4.38-4.28 (m, 1H), 4.13 (s, 1H), 4.03-3.91 (m, 1H), 3.86-3.73 (m, 1H), 3.69 (dd, \ J = 9.8, 2.8 Hz, 1H), 3.61 (dd, \ J = 9.8, 3.6 Hz, 1H), 3.51 (dd, \ J = 9.8, 6.4 Hz, 1H). \\
\text{13C NMR (100 MHz, CDCl}_3) & \delta 153.31, 151.62, 137.59, 137.45, 137.30, 137.10, 134.16, 132.27, 130.96, 128.88, 128.82, 128.62, 128.57, 128.44, 128.26, 128.14, 128.08, 127.94, 126.91, 126.28, 126.02, 125.16, 120.56, 119.23, 81.67, 79.99, 73.50, 73.33, 72.58, 72.13, 71.10, 68.43, 65.61, 51.99. HRMS (ESI): m/z [M+H]+ calcd for C\textsubscript{46}H\textsubscript{48}N\textsubscript{5}O\textsubscript{6}: 722.3230, found: 722.3229.
\end{align*}
O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-4-Phenoxyphenylcarbamate (18) A white solid (115.5 mg, 84%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.43 (s, 1H), 7.45 (d, $J = 8.2$ Hz, 2H), 7.40-7.24 (m, 20H), 7.13 (d, $J = 4.4$ Hz, 2H), 7.08 (d, $J = 7.6$ Hz, 1H), 7.00 (t, $J = 8.1$ Hz, 4H), 5.88 (s, 1H), 4.76 (d, $J = 12.0$ Hz, 1H), 4.64-4.43 (m, 6H), 4.34 (d, $J = 11.8$ Hz, 1H), 4.08 (s, 1H), 3.96 (s, 1H), 3.77 (s, 1H), 3.67 (d, $J = 9.6$ Hz, 1H), 3.62-3.55 (m, 1H), 3.49 (dd, $J = 9.2$, 6.8 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.73, 153.30, 152.83, 151.48, 137.56, 137.43, 137.31, 137.16, 132.97, 129.78, 128.60, 128.45, 128.12, 128.05, 127.96, 123.04, 121.45, 119.89, 118.37, 81.55, 79.93, 73.55, 73.31, 72.56, 72.03, 71.08, 68.39, 51.93. HRMS (ESI): m/z [M+H] + calcd for C$_{45}$H$_{65}$N$_2$O$_7$: 764.3336, found: 764.3315.

![Image](image1)

O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-2,4-Difluorophenylcarbamate (19) A white solid (87.8 mg, 69%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.96 (s, 1H), 7.42-7.06 (m, 21H), 6.96 (t, $J = 7.6$ Hz, 2H), 5.85 (s, 1H), 4.76 (d, $J = 12.0$ Hz, 1H), 4.50 (m, 6H), 4.33 (d, $J = 11.2$ Hz, 1H), 4.06 (s, 1H), 3.94 (s, 1H), 3.77 (s, 1H), 3.66 (d, $J = 8.8$ Hz, 1H), 3.58 (d, $J = 7.2$ Hz, 1H), 3.53-3.42 (m, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 159.33, 159.29, 156.83, 156.79, 153.00, 151.66, 137.60, 137.45, 137.30, 137.12, 128.65, 128.58, 128.45, 128.21, 128.09, 127.95, 127.65, 114.07, 111.99, 111.77, 81.53, 80.01, 73.30, 72.54, 72.00, 71.09, 68.40, 51.88. HRMS (ESI): m/z [M+H] + calcd for C$_{43}$H$_{59}$F$_2$N$_2$O$_7$: 708.2885, found: 708.2862.

![Image](image2)

O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-2-Chloro-6-(trifluoromethyl)phenylcarbamate (20) A white solid (108.7 mg, 78%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.40 (s, 1H), 8.66 (d, $J = 1.2$ Hz, 1H), 7.50 (d, $J = 8.4$ Hz, 1H), 7.40-7.20 (m, 20H), 7.14 (d, $J = 6.4$, 2.8 Hz, 2H), 4.78 (d, $J = 12.0$ Hz, 1H), 4.58-4.47 (m, 5H), 4.41 (d, $J = 11.6$ Hz, 1H), 4.34 (d, $J = 11.6$ Hz, 1H), 4.07 (s, 1H), 3.95-3.89 (m, 1H), 3.82-3.72 (m, 1H), 3.68 (dd, $J = 9.8$, 2.8 Hz, 1H), 3.56 (dd, $J = 9.6$, 3.6 Hz, 1H), 3.48 (dd, $J = 9.8$, 6.4 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 151.94, 151.92, 137.51, 137.36, 137.12, 136.92, 135.20, 129.49, 128.62, 128.53, 128.40, 128.25, 128.18, 128.13, 128.03, 127.93, 126.14, 120.59, 117.25, 81.41, 79.84, 73.29, 73.27, 72.51, 72.05, 71.14, 68.38, 51.88. HRMS (ESI): m/z [M+H] + calcd for C$_{45}$H$_{59}$ClF$_3$N$_2$O$_7$: 774.2598, found: 774.2564.

![Image](image3)

O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-4-(Methylthio)phenylcarbamate (21) A white solid (82.7 mg, 64%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.42 (d, $J = 8.8$ Hz, 2H), 7.39-7.23 (m, 20H), 7.13 (dd, $J = 6.4$, 2.8 Hz, 2H), 4.75 (d, $J = 12.0$ Hz, 1H), 4.75 (d, $J = 12.0$ Hz, 4H), 4.62-4.49 (m, 2H), 4.47 (dd, $J = 11.8$, 2.0 Hz, 1H), 4.34 (d, $J = 11.2$ Hz, 1H), 4.08 (s, 1H), 4.00-3.91 (m, 1H), 3.82-3.72 (m, 1H), 3.66 (dd, $J = 9.6$, 2.8 Hz, 1H), 3.58 (dd, $J = 9.6$, 3.6 Hz, 1H), 3.49 (dd, $J = 9.8$, 6.4 Hz, 1H), 2.46 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 152.52, 151.52, 137.57, 137.43, 137.31, 137.16, 135.14, 133.18, 130.95, 128.88, 128.57, 128.42, 128.29, 128.08, 128.02, 127.92, 120.25, 81.56,
HRMS (ESI): m/z [M+H] + calcd for C_{42}H_{43}N_{3}O_{6}S: 718.2951, found: 718.2951.

O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-1-Thienylcarbamate (22) A white solid (75.6 mg, 62%). ^1H NMR (400 MHz, CDCl _3) δ 7.45-7.21 (m, 9H), 7.20-7.05 (m, 2H), 6.89 (dd, J = 14.8, 4.4 Hz, 2H), 6.71 (d, J = 2.8 Hz, 1H), 5.84 (s, 1H), 4.74 (d, J = 12.0 Hz, 1H), 4.65-4.38 (m, 6H), 4.33 (d, J = 11.4 Hz, 1H), 4.06 (s, 1H), 4.00-3.86 (m, 1H), 3.66 (dd, J = 9.8, 2.8 Hz, 1H), 3.57 (dd, J = 9.6, 3.6 Hz, 1H), 3.48 (dd, J = 9.6, 6.4 Hz, 1H); ^13C NMR (100 MHz, CDCl _3) δ 152.53, 151.68, 139.40, 137.51, 137.37, 137.25, 137.06, 128.63, 128.58, 128.09, 128.02, 127.93, 124.72, 118.13, 113.40, 81.47, 79.93, 73.39, 73.28, 72.50, 71.99, 71.05, 68.28, 51.83, 29.73. HRMS (ESI): m/z [M+H] + calcd for C_{39}H_{39}N_{3}O_{6}S: 678.2638, found: 678.2629.

O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene)amino-Z-N-2,1,3-Benzothiadiazol-4-ylcarbamte (23) A white solid (95.8 mg, 73%). ^1H NMR (400 MHz, CDCl _3) δ 9.89 (s, 1H), 7.44-7.25 (m, 15H), 7.14 (dd, J = 6.4, 2.4 Hz, 2H), 5.91 (s, 1H), 4.87 (d, J = 12.0 Hz, 1H), 4.67 (d, J = 12.0 Hz, 1H), 4.62-4.28 (m, 7H), 4.22 (s, 1H), 3.98-3.92 (m, 1H), 3.83-3.75 (m, 1H), 3.69 (dd, J = 9.8, 2.8 Hz, 1H), 3.58 (dd, J = 9.8, 4.0 Hz, 1H), 3.49 (dd, J = 9.8, 6.4 Hz, 1H); ^13C NMR (100 MHz, CDCl _3) δ 154.99, 152.17, 152.13, 148.15, 137.58, 137.45, 137.24, 137.24, 137.14, 131.15, 129.80, 128.89, 128.64, 128.55, 128.43, 128.25, 128.16, 128.07, 127.94, 115.37, 113.88, 81.49, 79.76, 73.31, 72.60, 72.12, 71.37, 68.57, 52.06. HRMS (ESI): m/z [M+H] + calcd for C_{41}H_{31}N_{5}O_{6}S: 730.2699, found: 730.2693.

O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-adamantylcarbamate (24) A white solid (106.4 mg, 81%). ^1H NMR (400 MHz, CDCl _3) δ 7.39-7.23 (m, 18H), 7.14-7.09 (m, 2H), 6.34 (s, 1H), 5.76 (s, 1H), 4.73 (d, J = 12.0 Hz, 1H), 4.50 (m, 6H), 4.33 (d, J = 11.6 Hz, 1H), 4.01 (s, 1H), 3.91 (s, 1H), 3.70 (d, J = 7.2 Hz, 1H), 3.64 (d, J = 9.8 Hz, 1H), 3.54 (dd, J = 9.8, 4.0 Hz, 1H), 3.46 (dd, J = 9.6, 6.4 Hz, 1H), 3.11 (s, 3H), 2.02 (s, 6H), 1.70 (s, 6H); ^13C NMR (100 MHz, CDCl _3) δ 153.35, 150.64, 137.65, 137.40, 137.45, 137.24, 137.14, 131.15, 129.80, 128.89, 128.64, 128.55, 128.43, 128.25, 128.16, 128.07, 127.94, 115.37, 113.88, 81.49, 79.76, 73.31, 72.60, 72.12, 71.37, 68.57, 52.06. HRMS (ESI): m/z [M+H] + calcd for C_{45}H_{51}N_{3}O_{6}: 730.3856, found: 730.3860.

O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-tert-Butylcarbamate (25) A white solid (84.5 mg, 72%). ^1H NMR (400 MHz, CDCl _3) δ 7.56-7.22 (m, 20H), 7.18-7.02 (m, 2H), 4.73 (d, J = 12.0 Hz, 1H), 4.51 (m, 6H), 4.34 (d, J = 11.6 Hz, 1H), 4.02 (d, J = 2.4 Hz, 1H), 3.91 (dd, J = 4.0, 3.2 Hz, 1H), 3.77-3.68 (m, 1H), 3.65 (dd, J = 9.6, 2.4 Hz, 1H), 3.55 (dd, J = 9.6, 4.2 Hz, 1H), 3.47 (dd, J = 9.6, 6.4 Hz, 1H), 1.39 (s, 9H); ^13C NMR (100 MHz, CDCl _3) δ
O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-1-Dodecylcarbamate (26) A white solid (105.9 mg, 77%). ¹H NMR (400 MHz, CDCl₃) δ 7.41-7.24 (m, 18H), 7.15-7.09 (m, 2H), 6.44 (t, J = 5.6 Hz, 1H), 5.78 (s, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.61-4.41 (m, 6H), 4.33 (d, J = 11.6 Hz, 1H), 4.01 (s, 1H), 3.92 (d, J = 2.8 Hz, 1H), 3.72 (dd, J = 9.2, 5.6 Hz, 1H), 3.66-3.60 (m, 1H), 3.55 (dd, J = 9.8, 3.6 Hz, 1H), 3.47 (dd, J = 9.8, 6.4 Hz, 1H), 3.32-3.23 (m, 2H), 1.62-1.51 (m, 2H), 1.29 (m, 18H), 0.88 (t, J = 6.8 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 155.86, 151.03, 137.62, 137.46, 137.36, 137.26, 128.53, 128.40, 128.07, 128.01, 127.89, 81.72, 79.95, 73.64, 73.28, 72.56, 71.92, 70.91, 68.43, 51.86, 41.16, 31.97, 29.88, 29.69, 29.41, 26.93, 22.75, 14.21. HRMS (ESI): m/z [M+H]+ calcd for C₇₄H₅₂N₅O₆: 652.3387, found: 652.3395.

General procedure for removal Benzyl protective groups
Each compound of 15-26 (50 mg) was dissolved in 5 mL dry CH₂Cl₂ at -78°C for 20 min, then 2.5 mL BCl₃ (1M in hexane) was added and continually stirred at -78°C for 3 h and then 0°C for 12 h. The mixture was moved to warm and concentrated in vacuo and the crude product purified by flashing column chromatography (EtOAc/acetone/NH₄OH/H₂O = 8:2:0.5:0.5) to get the target product.

Data for O-(D-glucopyranosylidene) amino-Z-N-phenylcarbamate (28). A colorless oil (19.0 mg, 82%). ¹H NMR (400 MHz, CD₂OD) δ 3.30-3.25 (m, 1H), 3.51 (t, J = 8.8 Hz, J = 18 Hz,1H), 3.62 (dd, J = 6.8 Hz, J = 11.2 Hz,1H), 3.68 (t, J = 8.8 Hz, 1H), 3.94 (dd, J = 2.8 Hz, J = 11.2 Hz, 1H), 4.21 (d, J = 8.8 Hz, 1H), 7.07 (t, J = 7.2 Hz, J = 14.4 Hz, 1H), 7.33 (t, J = 7.6 Hz, J = 15.6 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H); ¹³C NMR (100 MHz, CD₂OD) δ: 155.52, 153.95, 137.89, 128.52, 123.48, 119.32, 75.12, 69.39, 61.96, 57.92; HRMS (ESI): m/z [M+H]+ calcd for C₁₀H₂₁NO₄: 312.1190, found: 312.1192.
for C$_{19}$H$_{21}$N$_3$O$_7$: 362.1352, found: 362.1350.

Data for O-(D-glucopyranosylidene) amino-Z-N-4-Phenoxyphenylcarbamate (30). A colorless oil (20.6 mg, 78%).
$^1$H NMR (400 MHz, CDOD$_3$) δ 7.52 (d, $J = 8.8$ Hz, 2H), 7.34 (t, $J = 8.0$ Hz, 2H), 7.18 (dd, $J = 12.8$, 7.6 Hz, 1H), 7.09 (t, $J = 7.2$ Hz, 1H), 6.97 (d, $J = 8.8$ Hz, 3H), 4.21 (d, $J = 8.8$ Hz, 1H), 3.94 (dd, $J = 11.2$, 2.8 Hz, 1H), 3.72 - 3.59 (m, 2H), 3.52 (t, $J = 8.8$ Hz, 1H), 3.27 (m, 1H).$^{13}$C NMR (100 MHz, CDOD$_3$) δ 157.73, 155.46, 154.20, 153.28, 133.48, 129.45, 122.75, 121.12, 119.11, 118.13, 117.97, 75.12, 69.41, 61.98, 57.95. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{19}$H$_{21}$N$_3$O$_7$: 404.1458, found: 404.1455.

Data for O-(D-glucopyranosylidene) amino-Z-N-2,4-Difluorophenylcarbamate (31). A colorless oil (19.8 mg, 81%).
$^1$H NMR (400 MHz, CDOD$_3$) δ 7.35 (m, 1H), 7.07 (t, $J = 8.0$ Hz, 2H), 4.20 (d, $J = 8.4$ Hz, 1H), 3.93 (dd, $J = 11.2$, 6.8 Hz, 1H), 3.68 (t, $J = 8.4$ Hz, 1H), 3.62 (dd, $J = 11.2$, 6.8 Hz, 1H), 3.52 (t, $J = 8.8$ Hz, 1H), 3.29 (m, 1H); $^{13}$C NMR (100 MHz, CDOD$_3$) δ 159.86, 159.82, 157.38, 157.33, 155.63, 154.88, 128.21, 128.12, 128.02, 111.48, 111.43, 111.30, 111.25, 75.11, 69.49, 69.39, 61.92, 57.88. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{13}$H$_{15}$F$_2$N$_3$O$_6$: 348.1007, found: 348.1009.

Data for O-(D-glucopyranosylidene) amino-Z-N-2-Chloro-6-(trifluoromethyl) phenylcarbamate (32). A brown oil (22.4 mg, 84%).
$^1$H NMR (400 MHz, CDOD$_3$) δ 8.45 (s, 1H), 7.66 (d, $J = 8.4$ Hz, 1H), 7.41 (d, $J = 8.0$ Hz, 1H), 4.20 (d, $J = 7.6$ Hz, 1H), 3.94 (d, $J = 10.4$ Hz, 1H), 3.76-3.62 (m, 2H), 3.56 (t, $J = 8.4$ Hz, 1H), 3.35 (d, $J = 15.2$ Hz, 1H).$^{13}$C NMR (100 MHz, CDOD$_3$) δ 156.20, 153.24, 135.35, 130.01, 129.76, 127.65, 125.05, 122.35, 120.95, 117.90, 75.09, 69.70, 69.44, 61.75, 57.77. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{42}$H$_{40}$ClF$_3$N$_3$O$_6$: 414.0680, found: 414.0673.

Data for O-(D-glucopyranosylidene) amino-Z-N-4-(Methylthio)phenylcarbamate (33). A colorless oil (21.1 mg, 85%).
$^1$H NMR (400 MHz, CDOD$_3$) δ 7.49 (d, $J = 8.4$ Hz, 1H), 7.27 (d, $J = 8.8$ Hz, 1H), 4.19 (d, $J = 8.4$ Hz, 1H), 3.93 (dd, $J = 11.2$, 2.8 Hz, 1H), 3.71-3.55 (m, 1H), 3.51 (t, $J = 8.9$ Hz, 1H), 3.29-3.22 (m, 1H), 2.47 (s, 1H); $^{13}$C NMR (100 MHz, CDOD$_3$) δ 155.48, 153.91, 135.57, 133.22, 127.57, 119.94, 75.12, 69.39, 61.95, 57.91, 15.20. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{14}$H$_{19}$N$_3$O$_6$S: 358.1073, found: 358.1073.

Data for O-(D-glucopyranosylidene) amino-Z-N-1-Thienylcarbamate (34). A red oil (17.3 mg, 74%).
$^1$H NMR (400 MHz, CDOD$_3$) δ 6.94 (d, $J = 5.3$ Hz, 1H), 6.86 (t, $J = 4.4$ Hz, 1H), 6.78 (s, 1H), 4.20 (d, $J = 8.8$ Hz, 1H), 3.93 (d, $J = 11.2$ Hz, 1H). $^{13}$C NMR (100 MHz, CDOD$_3$) δ 155.48, 153.91, 135.57, 133.22, 127.57, 119.94, 75.12, 69.39, 61.95, 57.91, 15.20. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{14}$H$_{19}$N$_3$O$_6$S: 358.1073, found: 358.1073.
Data for O-(D-glucopyranosylidene) amino-Z-N-2,1,3-Benzothiadiazol-4-ylcarbamate (35). A yellow oil (20.5 mg, 81%). $^1$H NMR (400 MHz, CDOD$_3$) $\delta$ 8.04 (dd, $J$ = 6.8, 1.2 Hz, 1H), 7.59 - 7.48 (m, 2H), 4.13 (d, $J$ = 7.6 Hz, 1H), 3.85 (dd, $J$ = 11.2, 2.8 Hz, 1H), 3.64 (t, $J$ = 8.0 Hz, 1H), 3.57 (dd, $J$ = 11.2, 6.4 Hz, 1H), 3.46 (t, $J$ = 8.4 Hz, 1H), 3.29-3.23 (m, 1H). $^{13}$C NMR (100 MHz, CDOD$_3$) $\delta$ 156.33, 154.91, 153.04, 147.83, 130.47, 129.73, 115.08, 114.04, 75.18, 69.84, 69.50, 61.79, 57.67. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{13}$H$_{15}$N$_3$O$_5$: 370.0821, found: 370.0824.

Data for O-(D-glucopyranosylidene) amino-Z-N-adamantylcarbamate (36). A colorless oil (18.5 mg, 73%). $^1$H NMR (400 MHz, CDOD$_3$) $\delta$ 4.01 (d, $J$ = 8.8 Hz, 1H), 3.79 (dd, $J$ = 11.2, 2.8 Hz, 1H), 3.56-3.41 (m, 2H), 3.35 (t, $J$ = 8.8 Hz, 1H), 3.15-3.01 (m, 1H), 1.98 (s, 3H), 1.92 (s, 6H), 1.63 (s, 6H); $^{13}$C NMR (100 MHz, CDOD$_3$) $\delta$ 154.89, 154.77, 75.16, 69.50, 69.44, 62.01, 57.86, 50.71, 41.10, 36.02, 29.55. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{17}$H$_{27}$N$_3$O$_6$: 370.1978, found: 370.1974.

Data for O-(D-glucopyranosylidene) amino-Z-N-tert-Butylcarbamate (37). An orange oil (18.6 mg, 83%). $^1$H NMR (400 MHz, CDOD$_3$) $\delta$ 4.01 (d, $J$ = 8.4 Hz, 1H), 3.80 (dd, $J$ = 11.2, 2.8 Hz, 1H), 3.49 (dt, $J$ = 11.2, 7.6 Hz, 2H), 3.36 (t, $J$ = 8.8 Hz, 1H), 3.12 (m, 1H), 1.27 (s, 9H); $^{13}$C NMR (100 MHz, CDOD$_3$) $\delta$ 155.23, 154.87, 75.20, 69.50, 69.44, 62.01, 57.86, 50.24, 27.60. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{11}$H$_{21}$N$_3$O$_6$: 292.1509, found: 292.1508.

Data for O-(D-glucopyranosylidene) amino-Z-N-1-Dodecylcarbamate (38). A white solid (22.4 mg, 85%). $^1$H NMR (400 MHz, CDOD$_3$) $\delta$ 4.13 (d, $J$ = 8.8 Hz, 1H), 3.91 (dd, $J$ = 11.2, 2.8 Hz, 1H), 3.60 (dt, $J$ = 11.2, 7.6 Hz, 2H), 3.48 (t, $J$ = 8.8 Hz, 1H), 3.22 (dt, $J$ = 11.6, 5.2 Hz, 3H), 1.60-1.52 (m, 2H), 1.33 (d, $J$ = 13.2 Hz, 18H), 0.92 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, CDOD$_3$) $\delta$ 157.33, 154.91, 75.18, 69.37, 61.94, 57.87, 40.58, 31.68, 29.36, 29.08, 26.47, 22.34, 13.05. HRMS (ESI): m/z [M+H]$^+$ calcd for C$_{19}$H$_{37}$N$_3$O$_6$: 404.2761, found: 404.2763.

Recombinant GCase (imiglucerase, Cerezyme) inhibition assay

Recombinant GCase was obtained from Genzyme (Cambridge, MA). Imiglucerase in vitro activity was determined with 4-MU-β-D-Glu as the substrate in McIlvaine buffer (100 mM sodium citrate, pH 5.2 or 200 mM sodium phosphate buffer, pH 7.0) containing sodium taurocholate (0.25% w/v) and Triton X-100 (0.1% v/v). To assay IC$_{50}$...
enzyme solution (12.5 µL, 0.1 mg/mL) without (control) or with inhibitor (7.5 µL, various concentrations) were incubated at 37°C for 30 min. Then, substrate (30 µL, 4.0 mM, McIlvaine buffer, pH 5.2 or 7.0) was added, and the samples were incubated for another 10 min at 37°C. Enzymatic reactions were stopped by adding of aliquots 150 µL of 100 mM glycine/NaOH buffer (pH 10.6). The amount of 4-methylumbelliferon formed was determined with a fluorometer at 355 nm (excitation) and 460 nm (emission). IC₅₀ values were determined by plotting percent activity versus log [I] with eight different inhibitors concentrations. The type of inhibition and constant Kᵢ values were determined by Lineweaver-Burk or Dixon plots of assays performed with different concentrations of inhibitor and substrate. Lineweaver-Burk double reciprocal plots of compounds 27-38 were shown below:
Compound 32

\[ \frac{1}{[S]}(\text{mM}^{-1}) \]

Compound 33

\[ \frac{1}{[S]}(\text{mM}^{-1}) \]
Compound 36

Compound 37

Compound 38
Cell lines and culture
Gaucher lymphoblasts cell lines homozygous for the N370S GCase (GM10873) and wild-type CCC-ESF-1 fibroblasts were obtained from the National Platform of Experimental Cell Resources for Sci-Tech (Beijing, China). The lymphoblasts were cultured in RPMI-1640 medium (Invitrogen) contained with 15% FBS and 1% penicillin/streptomycin. The wild-type CCC-ESF-1 fibroblasts were cultured in DMEM (Invitrogen) contained with 10% FBS and 1% penicillin-streptomycin. All cell lines were maintained in a humidified atmosphere containing 5% CO₂ at 37°C.

Measurement of N370S GCase activity in lymphoblasts derived from patients with GD
Patient fibroblasts homozygous for the N370S mutation were seeded at a density of 5×10⁵ cells per well in 12-well plates for 24h. Then cells were incubated with or without various concentrations of compounds from 100 to 3.25 µM for 3 days. The cells were washed with PBS twice and lysed in 0.1 M citrate phosphate buffer (0.4% v/v sodium taurocholate and 0.4% Triton X-100) for 30 min at 4°C. Total protein was determined with a BCA protein assay kit (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China) referred to the manufacturer’s instructions. Sample (30 mg) and 4-MU-β-D-Glu (5.0 mM) were incubated in 0.1M pH 5.2 citrate phosphate buffer (0.25% sodium taurocholate and 0.1 % Triton X-100) at 37°C for 2 h. The reactions were stopped by adding 150 µL glycine/NaOH buffer (200 mM, pH 10.6) and fluorescence was measured (excitation wavelength 355 nm, emission wavelength 460 nm) with a fluorimeter. Nonspecific GCase activity was evaluated by addition of 2.5 mM Conduritol B Epoxide (Enzo Life Sciences, Ann Arbor, MI) to control wells and identified not more than 2% (in control cells). All experiments were performed in triplicate.

Cytotoxicity assay in wild-type human fibroblasts
CCC-ESF-1 cells were seeded at a density of 4000 cells per well in 96-well plates and incubated overnight. The cells were treated with a range of concentrations from 100 to 3.125 µM at 37°C in 5% CO₂ for 24h. Then each well was added 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT, 20 µL, 5 mg/mL in PBS; Solarbio) and the mixtures were incubated for additional at 37°C for 4 h. After removal of the medium, DMSO (150 µL) was added and quantified by microplate reader at 570 nm.

Molecular docking study
The AutoDock 4.0 package was used to further evaluate the binding mode between 38 and GCase. The crystal structures of GCase was obtained from the Protein Data Bank (ID: 2V3E). At this stage, the structure of human GCase was preprocessed and prepared by deleting all of the water molecules, and hydrogen atoms were then added. Then a grid box was generated before docking. PDBQT files of targets and ligands were prepared using AutoDockTools. The center of the grid box was placed in the active site of GCase. A genetic algorithm (GA) was used to simulate ligand-receptor binding. The number of GA runs was 200. The step size parameters of quaternion and torsion were set to 200. For compound 38, 200 independent runs were performed. Default values were used for all other parameters.

$^1$H NMR spectrum of 14

$^{13}$C NMR spectrum of 14
$^1$H NMR spectrum of 15

$^{13}$C NMR spectrum of 15
$^1$H NMR spectrum of 16

$^{13}$C NMR spectrum of 16
$^1$H NMR spectrum of 17

$^{13}$C NMR spectrum of 17
\(^1\)H NMR spectrum of 18

\(^{13}\)C NMR spectrum of 18
$^1$H NMR spectrum of 19

$^{13}$C NMR spectrum of 19

$^1$H NMR spectrum of 20
$^{13}$C NMR spectrum of 20

$^1$H NMR spectrum of 21
$^{13}$C NMR spectrum of 21

$^1$H NMR spectrum of 22
$^{13}$C NMR spectrum of 22

$^1$H NMR spectrum of 23
$^{13}\text{C NMR spectrum of 23}$

$^1\text{H NMR spectrum of 24}$
$^{13}$C NMR spectrum of 24

$^1$H NMR spectrum of 25
$^{13}$C NMR spectrum of 25

$^1$H NMR spectrum of 26
$^{13}$C NMR spectrum of 26
$^1$H NMR spectrum of 27

$^{13}$C NMR spectrum of 27
$^1$H NMR spectrum of 28

$^{13}$C NMR spectrum of 28
\(^1\)H NMR spectrum of 29

\(^{13}\)C NMR spectrum of 29
$^1$H NMR spectrum of 30

$^{13}$C NMR spectrum of 30
$^1$H NMR spectrum of 31

$^{13}$C NMR spectrum of 31
$^1$H NMR spectrum of 32

$^{13}$C NMR spectrum of 32
$^1$H NMR spectrum of 33

$^{13}$C NMR spectrum of 33
$^1$H NMR spectrum of 34

$^{13}$C NMR spectrum of 34
$^1$H NMR spectrum of 35

$^{13}$C NMR spectrum of 35

$^1$H NMR spectrum of 36
\textsuperscript{13}C NMR spectrum of 36

\textsuperscript{1}H NMR spectrum of 37
$^{13}$C NMR spectrum of 37

$^1$H NMR spectrum of 38
$^{13}$C NMR spectrum of 38